Zygotic Genome Activation During the Maternal-to-Zygotic Transition

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Abstract

Embryogenesis depends on a highly coordinated cascade of genetically encoded events. In animals, maternal factors contributed by the egg cytoplasm initially control development, whereas the zygotic nuclear genome is quiescent. Subsequently, the genome is activated, embryonic gene products are mobilized, and maternal factors are cleared. This transfer of developmental control is called the maternal-to-zygotic transition (MZT). In this review, we discuss recent advances toward understanding the scope, timing, and mechanisms that underlie zygotic genome activation at the MZT in animals. We describe high-throughput techniques to measure the embryonic transcriptome and explore how regulation of the cell cycle, chromatin, and transcription factors together elicits specific patterns of embryonic gene expression. Finally, we illustrate the interplay between zygotic transcription and maternal clearance and show how these two activities combine to reprogram two terminally differentiated gametes into a totipotent embryo.

Contents

INTRODUCTION

Maternal-to-zygotic transition (MZT): the period during embryogenesis when developmental control transitions from maternally provided factors to ones produced by zygotic (embryonic) transcription

Totipotency/ pluripotency: the capacity of a cell to give rise to all (totipotent) or most (pluripotent) differentiated cells in an organism

Maternal clearance: the process of regulated degradation of maternally provided RNAs and proteins during the MZT

Embryogenesis begins with a single cell composed of cytoplasm from the egg and nuclei from both parents that fuse to encode a single zygotic genome. How a fully formed organism arises, far removed in appearance and function from the zygote, has long been the subject of scientific inquiry. Efforts to understand the principles underlying organismal development were closely tied to experiments in the nineteenth century by Theodor Boveri and others, who used sea urchin embryos to investigate the relationship between cellular components and heredity (Laubichler & Davidson 2008). Cross-fertilization between gametes of different species was found to yield larvae with intermediate characteristics of both parents, suggesting that genetic determinants were encoded in the nuclear material contributed by the sperm. However, a range of hybrid characteristics was also observed in crosses using mechanically produced anucleate eggs, implying that some degree of genetic contribution was also conferred by the maternal cytoplasm (Laubichler & Davidson 2008).

These observations have laid the groundwork for our current understanding of embryonic development, a process subject to both cytoplasmic and nuclear control. Initially, the embryo is transcriptionally quiescent, and development is directed exclusively by maternally provided proteins and RNAs from the egg cytoplasm. Subsequently, developmental control switches to the products of an activated nuclear genome during a period called the maternal-to-zygotic transition (MZT).

The MZT encompasses two major molecular activities, which together reprogram the terminally differentiated oocyte and sperm to totipotency. One is maternal clearance, the deletion of maternal instructions—mRNA and proteins—that are necessary for oocyte maturation, homeostasis, and the first stages of embryogenesis but become unnecessary or possibly deleterious as the embryo develops. The other is the installation of new zygotic instructions through gene expression,

a process that is activated by the maternal program and is called zygotic genome activation (ZGA). Together these two activities dramatically remodel the embryonic gene expression landscape and cellular identities, a process that will be revisited throughout development and adulthood as cells differentiate and regenerate.

Recent reviews have provided extensive treatments of the mechanisms that regulate maternal clearance (Barckmann & Simonelig 2013, Walser & Lipshitz 2011). Here, we focus on the activation of zygotic gene expression in animals, though similar processes also occur in plants (Baroux et al. 2008, Xin et al. 2012). In the first section, we highlight recent advances in measuring the onset of zygotic transcription and present them in the context of seminal discoveries in the genetic control of embryogenesis. Next, we consider the mechanisms that drive ZGA and describe the interplay between the cell cycle, chromatin, and transcription factors in regulating embryonic gene expression. Finally, we discuss the functional consequences of ZGA and show that maternal clearance and zygotic transcription are intimately linked and combine to give rise to a reprogrammed embryo.

MEASURING ZYGOTIC GENE EXPRESSION

The Developmental Context of Zygotic Genome Activation

In most animals, the maternal contribution directs a series of synchronized mitoses while maintaining a relatively constant volume as it forms a blastula. Subsequently, coordinated cell movements during gastrulation form distinct germ layers that specify the various tissues in the mature organism. Within this framework of embryogenesis, there is extensive variation in the timing and duration of these events among different species. The initial cell cycles of the amphibian *Xenopus* (35 min), zebrafish (15 min), and the fruit fly *Drosophila melanogaster* (8 min) are synchronized and proceed in rapid succession, with gastrulation occurring within hours of fertilization (Foe & Alberts 1983, Gerhart 1980, Kane & Kimmel 1993). Development is also rapid in the nematode *Caenorhabditis elegans*, which forms a 28-cell gastrula after 100 min of asymmetric divisions (**Figure 1**) (Sulston et al. 1983). In contrast, mouse and other mammals have relatively long cell cycles, with the first cleavage occurring approximately one day after fertilization (**Figure 1**) followed by gastrulation five to six days later (Oron & Ivanova 2012).

The requirement for zygotic transcription for embryogenesis to proceed is universal across animals. Upon transcriptional inhibition, zebrafish and *Xenopus* embryos will continue to divide but fail to undergo gastrulation (Kane et al. 1996, Newport & Kirschner 1982a). Similarly, the *C. elegans* embryo experiences extreme morphological defects without zygotic transcription, despite reaching 100 cells before arresting (Edgar et al. 1994), and *D. melanogaster*, which does not complete cytokinesis for the first 13 cell cycles, requires zygotic transcripts for cellularization to occur (Edgar et al. 1986, Merrill et al. 1988). In mouse, development proceeds no further than the second mitosis, commonly referred to as the 2-cell block (Goddard & Pratt 1983, Golbus et al. 1973, Warner & Versteegh 1974). In each of these organisms, ZGA occurs well before these defects arise, suggesting that zygotic transcription does not merely coincide with a requirement to replenish RNAs for homeostasis but also is essential to direct new developmental programs. Discovering the identity of these early zygotic RNAs is thus essential to understanding how development proceeds.

Distinguishing De Novo Zygotic Transcription from the Maternal Contribution

From the earliest studies of embryonic RNA content, it was clear that maternally deposited RNAs greatly outnumber zygotic transcripts—representing between 40% and 75% of all protein-coding

Zygotic genome activation (ZGA): the period during the MZT when the embryonic genome first begins to transcribe RNA

Chromatin: the physical structure of a chromosome, consisting of DNA bound by nucleosomes and other proteins

Transcription factor: a protein that binds to DNA sequences to regulate gene expression

Cellularization: the partitioning of a multinucleated cytoplasm (syncytium) into individual cells, as observed during *Drosophila melanogaster* embryogenesis
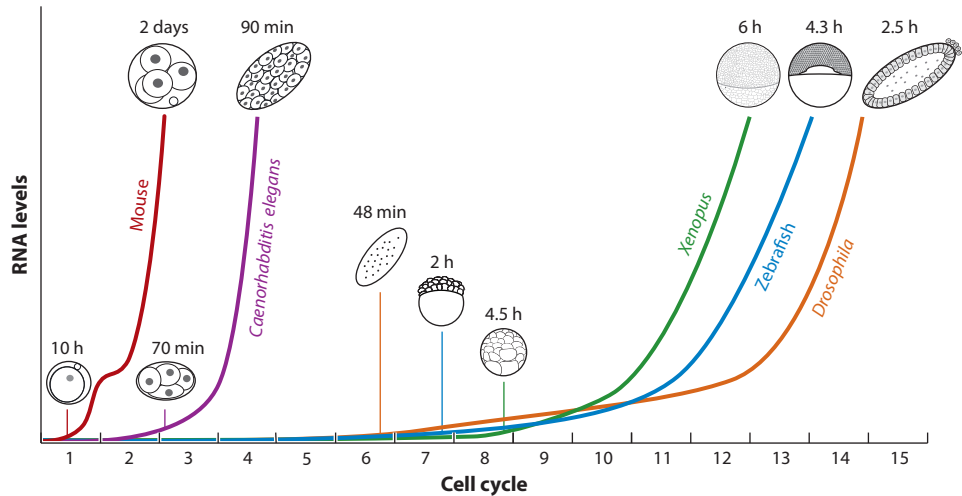
Figure 1

Timing of zygotic genome activation across various model organisms. Curves illustrate cumulative increases in zygotic gene expression as development progresses. In the mouse, a minor wave of transcription during the first cell cycle is followed by a second wave during cycle 2. *Caenorhabditis elegans* divisions are asynchronous, with transcription detected by four cells. Zygotic transcription in *Xenopus*, zebrafish, and *Drosophila melanogaster* is detected several cell cycles later and increases rapidly. Approximate times post fertilization are indicated.

genes across various species (Tadros & Lipshitz 2009, Wang et al. 2004, Wei et al. 2006) and still amounting to ∼60 to 70% of mRNA molecules at the peak of zygotic expression in zebrafish, for example (Lee et al. 2013) (**Figure 2a**). This large maternal contribution presents a challenge for detecting transcriptionally active genes, especially if maternal transcript copies greatly outnumber the zygotic contribution or if degradation of maternal copies occurs concurrently with de novo transcription, effectively canceling out the signal (De Renzis et al. 2007). This, coupled with dynamic regulation of maternal RNAs, including changes in poly(A) tail length, highlights the need to distinguish the maternal and zygotic contributions to the transcriptome pool.

Microarrays and high-throughput sequencing (RNA-Seq) using Illumina and SOLiD technologies (**Table 1**) have greatly enhanced our ability to dissect the maternal and zygotic RNA populations. Time-course experiments have revealed transcriptome-wide changes in gene expression during the MZT in many different species (Aanes et al. 2011, Collart et al. 2014, Dobson et al. 2004, Hamatani et al. 2004, Harvey et al. 2013, Heyn et al. 2014, Lee et al. 2013, Paranjpe et al. 2013, Sirard et al. 2005, Tan et al. 2013, Vassena et al. 2011, Vesterlund et al. 2011, Wang et al. 2004, Wei et al. 2006, Xie et al. 2010, Xue et al. 2013, Zhang et al. 2009); however, attributing these changes to active zygotic transcription can still be a challenge. Explicit techniques that distinguish bona fide zygotic gene expression from post-transcriptional regulation of the maternal contribution are thus invaluable for understanding the dynamics and extent of ZGA.

Four general approaches have been used to more accurately assess the scope of ZGA by emphasizing (**Figure 2b**) or removing (**Figure 2c**) the signal from zygotic transcripts. First, the maternal contribution can be depleted using subtractive hybridization techniques (**Figure 2d**). Zygotic samples hybridized to biotinylated oocyte cDNA can yield libraries where maternally contributed transcripts are underrepresented (Rothstein et al. 1992, Sive & St John 1988). This method has been effective for detecting rare zygotic transcripts from limited amounts of RNA (Zeng & Schultz 2003), but less so for accurate measurement of genome-wide transcript levels.

Transcriptome: the set of all RNA transcripts in a cell, tissue, or organism

RNA-Seq: massively parallel RNA sequencing to measure gene expression levels in a high-throughput manner
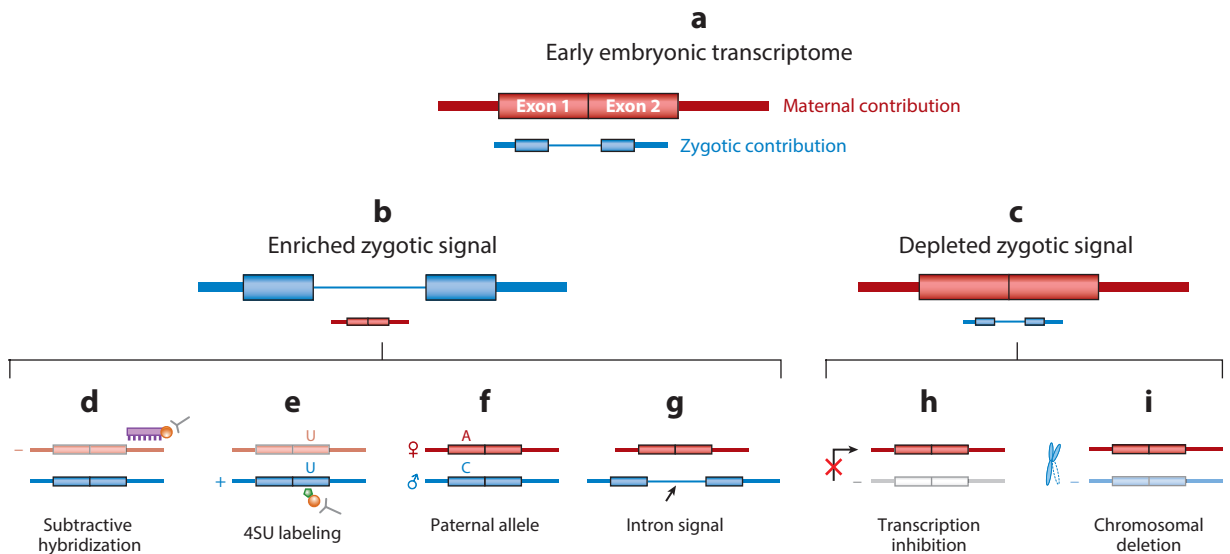
a
Early embryonic transcriptome

Maternal contribution
Zygotic contribution

b
Enriched zygotic signal

c
Depleted zygotic signal

d
Subtractive hybridization

e
4SU labeling

f
Paternal allele

g
Intron signal

h
Transcription inhibition

i
Chromosomal deletion

Figure 2

Identification of de novo zygotic transcription. (*a*) In the early embryo, the maternal contribution (*red*) represents the majority of the transcripts present. This can mask the relatively small amount of de novo zygotic transcription (*blue*) and lead to detection difficulties. Experimental techniques that (*b*) enrich or (*c*) deplete the signal from zygotic RNAs relative to the maternal contribution can be used to identify the genes that are de novo activated. (*d*) Subtractive hybridization employs biotinylated (*orange*) antisense oligos constructed from oocyte cDNA libraries (*purple*) to selectively deplete complementary maternal transcripts. (*e*) De novo transcribed mRNAs can be labeled using the nucleoside analog 4-thiouridine (4SU) (*green*) and pulled down by biotinylation (*orange*). (*f*–*g*) Zygotic transcripts can be identified by properties unique to the zygotic form, such as the presence of (*f*) the paternal genotype or (*g*) introns from pre-mRNAs, which should not be present in mature maternal mRNAs. (*h*) Transcription can be globally inhibited using chemicals such as α-amanitin. (*i*) Chromosomal deletion mutants remove the zygotic contribution for genes that fall within the deletion and can be used to identify genes that require both maternal and zygotic contributions to reach wild-type levels.

Second, the zygotic contribution can be depleted (**Figure 2c**). Chemical treatments, such as α-amanitin and actinomycin D (**Table 2**), applied early during embryogenesis globally inhibit RNA polymerase activity (**Figure 2h**). Thus, only changes in RNA levels that are sensitive to these treatments can be considered products of zygotic transcription (Edgar & Schubiger 1986, Edgar et al. 1994, Golbus et al. 1973, Hamatani et al. 2004, Kane et al. 1996, Lee et al. 2013, Newport & Kirschner 1982a, Vassena et al. 2011). Using this approach in combination with microarrays, Hamatani et al. (2004) were able to accurately measure the scope of distinct minor and major periods of zygotic transcription during mouse embryogenesis and showed that apparent expression-level increases in the early 1-cell stage were not due to transcription.

Selective loss of the zygotic contribution was achieved in *D. melanogaster* and *C. elegans* by measuring transcription in mutants with large chromosomal deletions (**Figure 2i**) (De Renzis et al. 2007, Merrill et al. 1988, Storfer-Glazer & Wood 1994). Systematic removal of entire chromosomal arms revealed many maternally provided genes whose levels decreased when their genomic region was absent, thus demonstrating that their wild-type levels are augmented by zygotically transcribed copies (De Renzis et al. 2007). In all, 20% of the *D. melanogaster* zygotic genome was found to contribute to embryonic development, two-thirds of which has a maternal contribution (De Renzis et al. 2007). These analyses helped to reveal shared mechanisms of activation that involve concomitant maternal clearance activity as well as transcriptional regulation via shared *cis* regulatory elements, both of which are further discussed below (De Renzis et al. 2007).

Table 1   High-throughput sequencing technologies used to interrogate the embryonic transcriptome and genome

Technique	Molecular target	Measures	Examples
RNA-Seq, poly(A)+	mRNA	Expression levels of polyadenylated (mature) mRNA	Aanes et al. 2011
RNA-Seq, total RNA	RNA	Expression levels of all RNAs (e.g., pre-mRNAs, introns); typically performed in combination with ribosomal RNA depletion	Lee et al. 2013, Paranjpe et al. 2013
Ribosome profiling	Coding mRNA	Translation efficiency of mRNAs based on sequencing of protected footprints of active ribosomes	Bazzini et al. 2012, Lee et al. 2013
CAGE-Seq	mRNA	Gene promoter/transcription start site based on sequencing of mRNA fragments at the 5′ m7G cap	Haberle et al. 2014
ChIP-Seq	DNA	Chromatin binding sites for specific proteins of interest, e.g., modified histones or transcription factors	Akkers et al. 2009, Harrison et al. 2011
DNase-Seq	DNA	Chromatin binding sites for all proteins based on digestion of free DNA (DNase I hypersensitivity)	Harrison et al. 2011, Thomas et al. 2011
MNase-Seq	DNA	Nucleosome positions on chromatin, based on digestion of surrounding DNA with MNase	Zhang et al. 2014
Bisulfite-Seq	DNA	Methylated and unmethylated cytosines in the genome, by assaying conversion to uracil by bisulfite treatment	Jiang et al. 2013, Potok et al. 2013

Zygotic genes can also be distinguished according to how they are regulated. Genes that are directly activated by the maternal contribution can be seen as a first wave of transcription, compared with genes that depend on factors encoded by the zygotic genome for their expression. In zebrafish, inhibitors of zygotic protein activity such as cycloheximide have been used to identify first-wave genes (Harvey et al. 2013, Lee et al. 2013) (**Table 2**). Lee et al. (2013) additionally used antisense inactivating morpholinos directed against spliceosomal RNAs to inhibit splicing and

Table 2   Chemical treatments used to study embryogenesis and zygotic genome activation

Treatment	Target of inhibition	Examples
Actinomycin D	Transcription (Pol I, II, III)	Golbus et al. 1973
α-Amanitin	Transcription (Pol II, III)	Golbus et al. 1973, Hamatani et al. 2004, Newport & Kirschner 1982a
Aphidicolin	DNA replication	Aoki et al. 1997, Wiekowski et al. 1991
5-Azadeoxycytidine	DNA methylation	Potok et al. 2013
Butyrate	Histone deacetylases	Adenot et al. 1997, Wiekowski et al. 1993
Cordycepin (3′-deoxyadenosine)	mRNA polyadenylation	Aanes et al. 2011, Aoki et al. 2003, Collart et al. 2014
Cycloheximide	Translation; cell cycle	Edgar & Schubiger 1986, Harvey et al. 2013, Kimelman et al. 1987, Lee et al. 2013
Cytochalasins	Actin polymerization; cytokinesis	Davis et al. 1996, Newport & Kirschner 1982a
Nocodazole	Microtubule polymerization; mitosis	Kimelman et al. 1987
Trichostatin A	Histone deacetylases	Adenot et al. 1997, Bultman et al. 2006
U1 and U2 antisense morpholinos	Spliceosome	Lee et al. 2013

maturation of zygotic transcripts, which led to the identification of 269 genes directly activated by maternal factors, constituting the first layer of zygotic gene transcription.

Third, the zygotic contribution can be labeled using modified ribonucleotides. Early on, radioactive nucleotide incorporation using ^{3}H or ^{32}P was widely used to measure zygotic genome activity. Newly produced radiolabeled RNA could be detected as early as the first two divisions in the sea urchin embryo (Nemer 1963, Poccia et al. 1985), during late cleavage stages in *Xenopus* (Brown & Littna 1964, Newport & Kirschner 1982a), at the blastoderm stage (2 h post fertilization) in *D. melanogaster* (Edgar & Schubiger 1986, Zalokar 1976), and at the 2-cell stage in mouse (Knowland & Graham 1972). Using bromo-uridine triphosphate, transcription was detected even earlier, mainly deriving from the male pronucleus in the 1-cell mouse embryo (Aoki et al. 1997, Bouniol et al. 1995). These analyses revealed general characteristics of the first zygotic genes based on molecular weight, which include heterogeneous mRNAs, as well as ribosomal RNA (Edgar & Schubiger 1986) and small RNAs transcribed by RNA polymerase III (Knowland & Graham 1972, Newport & Kirschner 1982a). However, determining the individual identity of transcribed genes using these techniques was a challenge.

Nucleoside analogs are more amenable to recovering gene identity. Heyn et al. (2014) injected 4-thio-uridine triphosphate into 1-cell zebrafish embryos, which was incorporated into new transcripts and selectively biotinylated at the thiol moieties (**Figure 2e**). Pull down with Illumina sequencing revealed enrichment of 592 genes transcribed in the early blastula, when cell cycle progression is still rapid. Accordingly, these genes are short, presumably to allow transcription to transpire in the limited period between rapid cell divisions (Heyn et al. 2014), a property that was also found in early *D. melanogaster* genes (De Renzis et al. 2007, Rothe et al. 1992). In addition, transcription by mitochondrial RNA polymerase was detected immediately after fertilization, indicating that the mitochondrial genome does not have a quiescent period, a result that echoes observations of cytoplasmic RNA synthesis in early sea urchin embryos (Craig 1970, Selvig et al. 1970) and may account for some of the signal observed in the early radiolabeling experiments.

Finally, sensitive RNA-Seq techniques can detect features of zygotic transcripts that distinguish them from the maternal contribution. When present, alternative splicing (Aanes et al. 2013), transcription start sites (TSSs) (Haberle et al. 2014, Nepal et al. 2013), and polyadenylation (Ulitsky et al. 2012) may generate zygotic-specific transcript isoforms for a gene. In the absence of such isoform differences, allelic information can be used. In most cases de novo transcription is expected to arise from both the maternal and paternal alleles, while the maternal contribution should consist of only maternal alleles. Assuming minimal RNA contribution from sperm, the appearance of single-nucleotide polymorphisms (SNPs) specific to the paternal genome can be used to gauge the activity of the zygotic genome (**Figure 2f**) (Sawicki et al. 1981). Using this approach, Harvey et al. (2013) performed RNA-Seq on embryos generated from two different zebrafish strains in parallel with whole-exome sequencing on the parental genomes. They found that 61% of expressed genes with informative SNPs had transcripts bearing both parental genotypes, showing that a large proportion of the embryonic transcriptome has both a maternal and zygotic contribution. Similar approaches were used to identify genes transcribed in early *D. melanogaster* embryos (Ali-Murthy et al. 2013, Lott et al. 2011) and to measure parent-of-origin gene expression by following the maternal and paternal genotypes in single-cell embryonic transcriptomes (Xue et al. 2013)—39% of polymorphic genes in the human 8-cell embryo displayed biallelic expression, which increased to 70% by the morula stage (Xue et al. 2013).

Because many genes do not contain informative polymorphisms, Lee et al. (2013) leveraged the capacity to sequence unspliced pre-mRNA molecules in ribosomal RNA–depleted total-RNA libraries, as an alternative to traditional poly(A)+ selected libraries (**Table 1**), which enrich for mature mRNAs (**Figure 2g**). Using intron sequence coverage as an unambiguous signal to

distinguish de novo zygotic transcription, they were able to identify >7,000 transcribed genes from the active zygotic genome in the late blastula, 74% of which had a maternal contribution that had previously obscured detection using exonic signal alone owing to low levels of expression (Lee et al. 2013). Thus, the scale of ZGA in zebrafish now appears to be at least an order of magnitude larger than has been reported previously, involving not only embryo-specific genes but also a large fraction of genes that were already represented in the maternal contribution (Harvey et al. 2013, Lee et al. 2013).

Comparisons between total and poly(A)+ sequencing libraries can also help distinguish de novo transcribed and polyadenylated RNAs from maternal RNAs that are subject to post-transcriptional regulation of the poly(A) tail. New zygotic expression is well correlated between the two sequencing strategies (Lee et al. 2013, Paranjpe et al. 2013). In contrast, maternal mRNAs with short poly(A) tails are less efficiently sequenced using poly(A)+ selection, resulting in apparent expression level differences owing solely to changes in poly(A) tail length (Collart et al. 2014, Paranjpe et al. 2013). In this way, cytoplasmic polyadenylation of maternal mRNAs was detected in early *Xenopus* embryos and distinguished from de novo zygotic transcription at a later stage, based on the asymmetry of total and poly(A)+ sequencing signals (Collart et al. 2014, Paranjpe et al. 2013). Together, these approaches have progressively uncovered earlier and more extensive activation of the zygotic genome and have identified the first genes that are transcribed during the MZT, providing insight into their mechanisms of coactivation.

Dynamics of Activation

The analyses described above have revealed extensive variability in the timing and dynamics of zygotic gene expression. Among vertebrates, mouse embryos experience the earliest ZGA with respect to cell cycle count (Aoki et al. 1997, Bouniol et al. 1995, Hamatani et al. 2004, Park et al. 2013, Xue et al. 2013). The punctuated bursts of zygotic transcription at 1-cell and 2-cell stages constitute a minor and major wave of ZGA (**Figure 1**) (Hamatani et al. 2004), now thought to involve as many as ~800 and ~3,500 genes, respectively (Park et al. 2013, Xue et al. 2013). These two waves of ZGA are in line with a global shift in chromatin organization spanning the first cleavage (see below in section on Chromatin Competency). Other mammals also seem to experience minor and major waves, with a trend toward slightly delayed expression patterns when compared with mouse (Dobson et al. 2004, Vassena et al. 2011, Xue et al. 2013, Zhang et al. 2009), though there is now evidence that human embryos are also transcriptionally active by the 1-cell stage (Xue et al. 2013), prior to the major wave at the 4–8-cell stages (Braude et al. 1988, Dobson et al. 2004, Vassena et al. 2011, Zhang et al. 2009).

In other vertebrates, peak zygotic genome activity occurs after several cell cycles have elapsed, but from an absolute time perspective this often occurs much sooner than in mammals. Lower levels of de novo transcribed genes appear earlier, though whether this constitutes a distinct early phase of activation rather than a gradual ramping up of transcription is unclear. In *Xenopus* embryos, high levels of transcription are observed at the midblastula transition (MBT) (see sidebar, The Mid-Blastula Transition), approximately 6–7 h and 12–13 cell cycles into development (Collart et al. 2014, Newport & Kirschner 1982a, Paranjpe et al. 2013, Tan et al. 2013), with early transcripts appearing between cycles 6 and 9 (**Figure 1**) (Blythe et al. 2010, Collart et al. 2014, Paranjpe et al. 2013, Yang et al. 2002). In zebrafish, multiple recent analyses place the onset of transcription between the 64-cell and 256-cell stages (cycles 7–9) (Aanes et al. 2011, Harvey et al. 2013, Heyn et al. 2014, Lee et al. 2013), starting with several hundred transcripts (Heyn et al. 2014) and increasing to several thousand prior to gastrulation (**Figure 1**) (Harvey et al. 2013, Lee et al. 2013). Previous observations of an earlier wave of activation at the 4-cell stage (Mathavan et al.

THE MID-BLASTULA TRANSITION

The mid-blastula transition (MBT) was originally defined with respect to amphibian development, and refers to the approximate midpoint of the blastula stage, after 12 cleavages in *Xenopus* (Gerhart 1980, Newport & Kirschner 1982a). At this stage, the cell cycle lengthens and becomes asynchronous, cells gain motility, and zygotic transcription is active and required, thus marking the time when nuclear control of the embryo begins. In this way, the MBT was the morphological embodiment of the MZT in *Xenopus*, as well as in other species such as zebrafish and *D. melanogaster* (Blankenship & Wieschaus 2001, Kane & Kimmel 1993). However, given that zygotic genome activity precedes the MBT in many organisms—including *Xenopus* (Skirkanich et al. 2011, Yang et al. 2002), but most notably mouse, which is already transcriptionally active at the 1-cell stage (Aoki et al. 1997, Bouniol et al. 1995, Hamatani et al. 2004, Park et al. 2013, Xue et al. 2013)—the MBT may not be such a widely applicable concept with respect to ZGA (Yasuda & Schubiger 1992).

2005) were likely due to cytoplasmic polyadenylation and not to zygotic transcription (Harvey et al. 2013).

The patterns in invertebrates are even more variable. In *D. melanogaster*, the bulk of zygotic transcription does not occur until the long cell cycle pause that accompanies cellularization (Benoit et al. 2009, De Renzis et al. 2007, Foe & Alberts 1983, Lécuyer et al. 2007), though transcription begins as early as cycle 6 (**Figure 1**) (Ali-Murthy et al. 2013, Karr et al. 1985). The expression dynamics leading up to cellularization remain to be resolved in a high-throughput manner. Among nematodes, *C. elegans* transcription levels increase steadily from the 4-cell stage until gastrulation, all within ~1.5 h (**Figure 1**) (Baugh et al. 2003, Edgar et al. 1994). This is in stark contrast to recent findings in the parasitic worm *Ascaris suum*, which remains at the 1-cell stage for 36 h and already has an active zygotic genome from fertilization, transcribing ~2,500 genes deriving from both pronuclei prior to fusion (Wang et al. 2014). Finally, sea urchin embryos also seem to be transcriptionally active at the 1-cell stage (Poccia et al. 1985), reaching a peak in the early blastula after 15 h (Wei et al. 2006).

These divergent patterns reveal that ZGA is regulated by multiple, diverse mechanisms that may be influenced by variable cell cycle dynamics and developmental contexts. Activation is widespread, though not ubiquitous, across the genome, suggesting that one component of ZGA may be a global attainment of genome competency, but with additional layers of regulation to account for gene-specific expression timing and magnitude. We examine many of these potential mechanisms in the following section.

MECHANISMS OF GENOME ACTIVATION

General Models of Activation

Traditionally, two contrasting models of activation have been the focus of research on ZGA. On the one hand, the nucleocytoplasmic (N/C) ratio model posits that the increasing quantity of nuclear material relative to a constant cytoplasm volume, through progressive cell divisions, alleviates transcriptional repression (Newport & Kirschner 1982a,b). Under this formulation, the barriers to ZGA are maternally provided repressive factors, whose relative levels must be diminished before transcription can occur (**Figure 3a**).

On the other hand, a maternal clock independent of the number of cell divisions may determine the timing of gene expression (Howe et al. 1995). The molecular instantiation of this model can

Nucleocytoplasmic (N/C) ratio: the ratio of nuclear to cytoplasmic volume in a cell

be seen as an increase in quantity or activity of a maternal factor, which must reach a critical level to trigger transcription (**Figure 3***b*). This model is particularly appealing, given the prevalence of cytoplasmic polyadenylation of maternally provided mRNAs (Aanes et al. 2011, Collart et al. 2014, Harvey et al. 2013, Richter & Lasko 2011) and correlated increases in translation efficiency, which can be seen through polysome profiling (Qin et al. 2007) and high-throughput ribosome footprinting (**Table 1**) (Lee et al. 2013). Thus, maternally provided mRNAs encoding critical components such as transcription factors and chromatin modifiers may be gradually mobilized over time.

These models are not mutually incompatible, or necessarily independent, and there is evidence in *D. melanogaster* that both modes of regulation exist simultaneously for the activation of roughly equal numbers of genes (Lu et al. 2009). However, it is also important to consider that all of these mechanisms also depend on the relative amount of DNA template available, which increases exponentially after each cell cycle. Reaching a threshold quantity of DNA, as well as allowing sufficient time for transcript numbers to accumulate, is a factor in achieving detectable levels of transcriptional output (**Figure 3***c,d*). The mechanisms that regulate ZGA may in fact act at an earlier time than when the effects can be measured using current techniques. In the following subsections, rather than focusing on these models, we explore control of ZGA from the perspective of three aspects of the developing embryo: the cell cycle; changes in chromatin structure, histone marks, and epigenetic prepatterning; and the activity of transcription factors.

Cell Cycle Regulation

In *Xenopus*, zygotic transcriptional activation accompanies the loss of cell cycle synchrony at the MBT (Gerhart 1980). In investigating the mechanisms that influence the timing of these events, Newport & Kirschner (1982a,b) discovered that an increasing N/C ratio controls the MBT and zygotic transcription, rather than the number of cleavages or rounds of DNA synthesis.

In a series of experiments that altered the N/C ratio using cleavage inhibitors (**Table 2**), mechanical constriction of the cytoplasm, induced polyspermy, and injections of exogenous nonspecific DNA, they found that transcription could be prematurely activated when the DNA content equaled that found in wild-type cycle 13 embryos, independent of cell cycle count (Newport & Kirschner 1982a,b). From these observations, Newport & Kirschner (1982a,b) proposed that titration of some maternally provided repressive factor, relative to an exponentially increasing amount of DNA, ultimately determined the timing of ZGA. Such factors could include heterochromatin-promoting histones or transcription inhibitors, both of which are discussed in the following subsections.

This role of the N/C ratio was subsequently observed in the zebrafish mutant *futile cycle* (*fue*), in which failure of chromosomal segregation leads to polyploid cells with locally higher N/C ratios and premature RNA polymerase II (Pol II) initiation (Dekens et al. 2003). Zygotic transcription in *D. melanogaster* embryos also depends on an increasing N/C ratio; however, it seemed to strongly affect only a subset of genes, suggesting that other mechanisms are in place to regulate ZGA (Edgar et al. 1986, Lu et al. 2009, Yasuda et al. 1991).

Other evidence suggests that the N/C ratio affects transcription activation indirectly, through regulation of cell cycle rate. Edgar et al. (1986) found that *D. melanogaster* mitotic cycle length prior to cellularization slows according to the N/C ratio. Recently, the Cdc25 homolog Twine has been implicated in effecting this cell cycle pause: Degradation of Twine, possibly in an N/C ratio–dependent manner, stabilizes Cdk1 phosphorylation, thus preventing entry into mitosis and allowing zygotic transcription to occur (**Figure 4***a*) (Di Talia et al. 2013, Farrell & O'Farrell 2013). However, early zygotic transcription prior to cellularization is in turn required for Twine
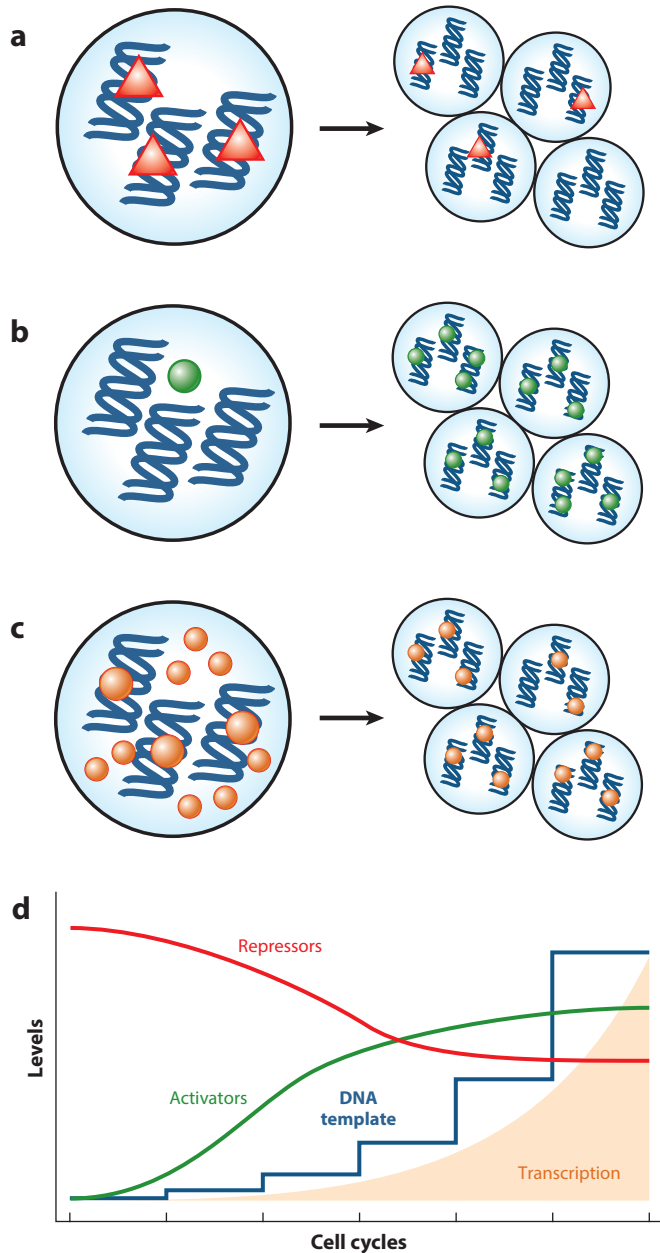
Figure 3

General models for zygotic genome activation. (*a–c*) Schematics illustrating the relationship between maternal factors and the zygotic genome as cells divide. (*a*) Early during embryogenesis, a hypothetical maternal repressor (*red*) prevents zygotic transcription. After several divisions, the repressor is titrated away as the ratio of nuclear material to cytoplasm increases (the nucleocytoplasmic ratio), thus allowing transcription to initiate. (*b*) Developmental time may be necessary for sufficient levels of an activator (*green*) to accumulate, e.g., resulting from activated translation of a maternal mRNA. (*c*) Alternatively, the embryo may already possess transcriptional activators (*orange*) and be transcriptionally competent early, but a threshold amount of DNA template is required for transcription to be detected. (*d*) All of these general mechanisms could combine to elicit increasing transcription levels over developmental time.
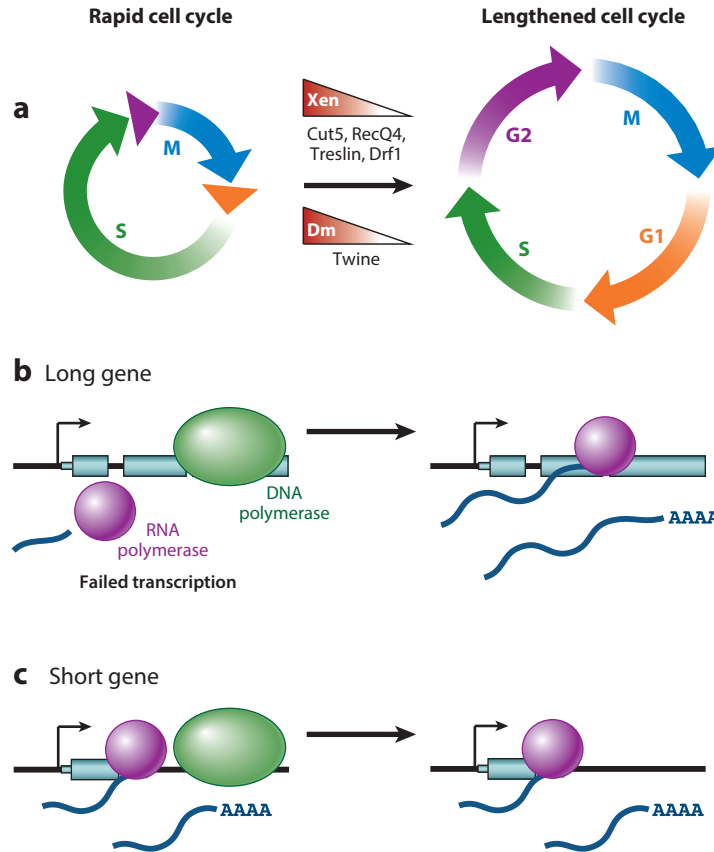
Figure 4

Influence of cell cycle on zygotic gene transcription. (*a*) In some species, early cleavage stages progress through mitosis (M) and DNA synthesis (S) phases with little to no gap phases (G1 and G2). As the embryo divides, maternal DNA replication factors—Cut5, RecQ4, Treslin, and Drf1 in *Xenopus* (Xen), and Twine in *Drosophila melanogaster* (Dm)—are titrated away as the nucleocytoplasmic ratio increases, resulting in cell cycle lengthening. (*b*) Rapid cell cycles may be incompatible with transcription of longer genes, resulting in failed or abortive RNA polymerase engagement due to interference with the DNA replication machinery (*left*). Longer cell cycles would allow more time for elongation and the accumulation of zygotic transcripts (*right*). (*c*) However, rapid cell cycles do not appear to be prohibitive for transcription of short genes.

degradation (Farrell & O'Farrell 2013, Sung et al. 2013), indicating that this mechanism cannot account for all of ZGA.

In *Xenopus*, the four replication factors Cut5, RecQ4, Treslin, and Drf1 have been found to control the cell cycle and thus guide the onset of zygotic transcription (Collart et al. 2013). Titration of these factors causes DNA replication to slow, leading to the onset of asymmetric cell divisions at the MBT (**Figure 4a**). Overexpression resulted in an increase in replication initiation and cell cycle count and as a result delayed the transcription of a large number of genes. Additionally, early activation of the replication checkpoint kinase Chk1 was observed (Collart et al. 2013). Chk1 regulates entry into S and G2 phases via repression of cyclin-dependent kinase activity (reviewed in Sørensen & Syljuåsen 2012); thus, together these mechanisms may contribute to the lengthening of the cell cycle at the *Xenopus* MBT and the associated increase in zygotic transcription.

Gap phase: the period between DNA replication (S) and mitosis (M) during the cell cycle; G1 follows M phase, G2 follows S phase

These results indicate that cell cycle length plays a role in at least the later stages of zygotic gene activation. *Xenopus*, zebrafish, and *D. melanogaster* all experience rapid, synchronous cell cycles during the first cleavages, in contrast to the slow pace of cell cycles in mouse. Congruently, the bulk of zygotic gene activation occurs in the former species only after the cell cycle begins to slow, whereas it has already occurred in the 2-cell mouse embryo. Consistent with this observation, chemical inhibition of either DNA replication or the cell cycle results in precocious expression of zygotic transcripts in *Xenopus* (**Table 2**) (Kimelman et al. 1987). Similarly, in *D. melanogaster*, interphase arrest induces premature transcription at cycle 10; however, earlier treatment inhibits transcription altogether, suggesting that a critical supply of protein activators is also necessary and that longer cell cycles are permissive rather than sufficient for transcription (Edgar & Schubiger 1986).

Thus, short cell cycles appear to be incompatible with the time it takes to transcribe and process longer genes, which would be prematurely disrupted by the DNA replication machinery (**Figure 4***b,c*). Three lines of evidence support this observation. First, in *D. melanogaster*, failure to complete transcription during early mitoses has been detected at individual gene loci using in situ nuclear labeling of mRNA 5′ and 3′ ends. Abortive transcription followed by degradation is detected for *ultrabithorax* until the G2 pause of cycle 14 (Shermoen & O'Farrell 1991). Second, key components of RNA biogenesis are inhibited during mitosis, including polyadenylation and splicing (Colgan et al. 1996, Shin & Manley 2002). Third, early-transcribed genes tend to be short. Rothe et al. (1992) showed that early expression of the gap gene *knirps* was possible owing to its short 3-kilobase (kb) length. In contrast, homologous *knirps-related* is 20 kb longer and was subject to abortive transcription. In an elegant demonstration of the significance of gene length, they were able to rescue the segmentation defects in *knirps* mutants using *knirps-related*, but only when expressed as an intronless minigene and not when the 19-kb intron was included (Rothe et al. 1992). Efficient and rapid splicing is required for normal *D. melanogaster* embryogenesis (Guilgur et al. 2014), and the trend toward shorter genes with fewer introns appears to be a general feature of the earliest transcripts across different organisms (De Renzis et al. 2007, Heyn et al. 2014, Swinburne & Silver 2008).

Taken together, cell cycle dynamics may define a hierarchy of zygotic gene activation, in which transcription of shorter genes is compatible with rapid cell cycles, whereas expression of longer genes is delayed until the cell cycle lengthens. In this way, the cell cycle length is permissive for ZGA, which implies that additional, instructive mechanisms are required for proper activation to occur.

Chromatin Competency

When Newport & Kirschner (1982b) microinjected a plasmid containing a yeast tRNA gene into fertilized *Xenopus* eggs, they found that the RNA was synthesized as soon as 10 min after injection, well before endogenous zygotic transcription takes place. Similar results were obtained in mouse (Wiekowski et al. 1993) and zebrafish (Harvey et al. 2013), and together they indicate that many components of the transcription complex are already competent in the early vertebrate embryo. However, Newport & Kirschner (1982b) went on to show that transcription of the plasmid was subsequently repressed, only to resume again in the late blastula. Because coinjection of nonspecific DNA was able to rescue expression, presumably by competing away the repression, they hypothesized that the effect was due to assembly of the plasmid DNA into closed chromatin.

The interplay between transcriptional machinery and chromatin in the early embryo likely regulates the timing of ZGA (Prioleau et al. 1994). Chromatin is composed of DNA wound around nucleosomes, octamers of the core histone proteins H2A, H2B, H3, and H4, which are in

turn joined together by histone linker proteins (e.g., H1) to form compacted heterochromatin. As transcription factors bind to regulatory regions in the DNA sequence, access to a particular gene locus can be occluded by this closed structure.

Chromatin accessibility is regulated through nucleosome position and configuration, which is influenced by histone variants as well as post-transcriptional modifications of histone N-terminal tails (marks), such as methylation and acetylation. In mouse, hyperaccessible chromatin seems to underlie ZGA (Cho et al. 2002). Prior to fusion into the zygotic nucleus, the uncondensed male pronucleus is transcriptionally competent, and low levels of endogenous transcription during mid to late S phase contribute to the minor wave of ZGA (Aoki et al. 1997, Bouniol et al. 1995, Park et al. 2013, Ram & Schultz 1993, Wiekowski et al. 1993, Xue et al. 2013). In contrast, the female pronucleus remains transcriptionally silent (Wiekowski et al. 1993). This asymmetry is likely due to a transient open chromatin state induced by the repackaging of the paternal genome. Sperm DNA is bound by arginine-rich protamines, which are exchanged for maternal histones prior to S phase (Nonchev & Tsanev 1990). These new histones are subject to a transcriptionally permissive pattern of modifications, including H4 hyperacetylation (Adenot et al. 1997, van der Heijden et al. 2006) and H3K9 and H3K27 monomethylation (Santos et al. 2005). Protamines, interspersed with paternal histones, are also found in human, *Xenopus*, and *D. melanogaster* sperm DNA (Hammoud et al. 2009, Jayaramaiah Raja & Renkawitz-Pohl 2005, Shechter et al. 2009), but surprisingly not in that of zebrafish (Wu et al. 2011).

Histone exchange is a general mechanism during embryogenesis, occurring as gamete-specific variants are replaced by somatic versions. This process could mediate gradual nucleosome unpacking prior to ZGA, as maternal histones are diluted in favor of permissive zygotic versions (**Figure 5a**). The repressive H2A variant macroH2A is found preferentially in the mouse female pronucleus and appears to contribute to its transcriptional silence; macroH2A is progressively lost as the embryo becomes transcriptionally active (Chang et al. 2005). In contrast, embryonic H2A.Z is required for development (Faast et al. 2001, Whittle et al. 2008). In *C. elegans*, H2A.Z (HTZ-1) was found to be translated from maternal mRNAs and incorporated proximal to developmentally critical genes, suggesting that it influences expression specificity (Whittle et al. 2008). H3.3 incorporation is required for male pronuclear formation in *D. melanogaster* (Torres-Padilla et al. 2006), whereas H3.3 knockdown in mouse induces chromosomal overcondensation by the 2-cell stage and impairs zygotic transcription in the morula (Lin et al. 2013). Finally, repressive oocyte-specific H1 linker histone variants are replaced by somatic versions coincident with the alleviation of transcriptional quiescence (Fu et al. 2003, Pérez-Montero et al. 2013, Smith et al. 1988, Tanaka et al. 2001). In *D. melanogaster*, loss of embryonic H1 variant dBigH1 leads to premature Pol II activity and gene expression (Pérez-Montero et al. 2013), showing that one barrier to ZGA is the early prevalence of higher-order chromatin.

Thus, a globally permissive chromatin conformation is a prerequisite for ZGA, which is shaped in part by dynamic incorporation of embryonic histone variants. However, the specificity of activation—i.e., the genes that are eventually transcribed from the competent genome—likely requires local changes to chromatin accessibility. In the following two subsections, we examine the roles that histone marks and epigenetic prepatterning may play in guiding these changes.

Histone Modifications

Two types of histone modifications have been implicated in shaping gene expression during the MZT: lysine acetylation and lysine (tri)methylation. In mouse, permissive H4 acetylation distinguishes the transcriptionally active male pronucleus from the silent female pronucleus (Adenot et al. 1997, van der Heijden et al. 2006). Following the minor wave of ZGA, histone deacetylase

activity contributes to a transient period of transcriptional repression, such that injected plasmids as well as endogenous genes that are normally expressed at the 1-cell stage are not readily transcribed in the 2-cell embryo (Martínez-Salas et al. 1989, Wiekowski et al. 1991). Inhibition of histone deacetylases (Davis et al. 1996) or DNA synthesis (**Table 2**) (Christians et al. 1995) relieves this repression, suggesting that replication-dependent deacetylation provides transcriptional specificity for the major wave of ZGA. Widespread H4 deacetylation leading up to the MBT has also been observed in *Xenopus* (Dimitrov et al. 1993), consistent with the creation of a default off transcriptional state coupled with gene-specific regulation of chromatin accessibility. Chromatin remodelers that induce or respond to acetylation are likely involved in this process. Two maternally provided components of the ATP-dependent chromatin remodeling SWI/SNF complex, Brg1 and SRG3, are required for mouse embryogenesis (Bultman et al. 2006, Sun et al. 2007), with loss of Brg1 resulting in reduced levels of 30% of zygotic genes and arrest at the 2-cell stage (Bultman et al. 2006).

H3 lysine methylation also influences the timing of gene activation during the MZT (Akkers et al. 2009, Chen et al. 2013, Lindeman et al. 2011, Schuettengruber et al. 2009, Vastenhouw et al. 2010), though its effects vary widely across different species and contexts. The opposing marks H3K4 and H3K27 trimethylation (H3K4me3 and H3K27me3) at gene promoter regions have received considerable attention for their association with active and repressed gene expression, respectively (**Figure 5b**). In mouse, the transcriptionally inactive female pronucleus is associated with H3K27me3, with the transcriptionally competent male pronucleus acquiring H3K27me3 only toward the end of the minor ZGA (Santos et al. 2005). However, H3K4me3 is also found preferentially in the female pronucleus despite its transcriptional quiescence, and this asymmetry rapidly diminishes as the male pronucleus incorporates maternal H3 histones (Lepikhov & Walter 2004). Thus, H3K27me3, but not H3K4me3, seems to affect early mouse gene activity.

In other organisms where large numbers of embryos are more readily available, high-throughput chromatin immunoprecipitation assays (**Table 1**) show greater association of H3K4me3 with transcription, but its role in guiding ZGA is not straightforward. In *D. melanogaster*, H3K4me3 becomes strongly enriched in zygotic-transcribed genes that have a maternal contribution (Chen et al. 2013, Schuettengruber et al. 2009), but only later in development. Neither H3K4me3 nor H3K27me3 is present prior to cellularization, even among early-expressed genes (Chen et al. 2013).

For both zebrafish and *Xenopus*, H3K4me3 is found across embryonic gene promoters, with a preference for those with housekeeping roles expressed soon after ZGA (Akkers et al. 2009, Lindeman et al. 2011, Vastenhouw et al. 2010). In contrast, H3K27me3 preferentially associates with genes encoding specific developmental functions (Lindeman et al. 2011, Vastenhouw et al. 2010) and subject to differential spatial regulation (Akkers et al. 2009). Thus, in these species, H3K4me3 and H3K27me3 appear to distinguish earlier versus later zygotic transcription.

However, H3K4me3 is also found on inactive genes in zebrafish embryos, based on lack of H3K36me3 signal (which marks Pol II elongation) or evidence of transcription in RNA-Seq experiments (Lindeman et al. 2011, Vastenhouw et al. 2010). These genes do tend to be expressed at later stages, suggesting that H3K4 trimethylation also marks promoters that are poised for rapid mobilization in the appropriate developmental context (Lindeman et al. 2011, Vastenhouw et al. 2010). In addition, many of these promoters are also simultaneously occupied by inactivating H3K27me3 (**Figure 5b**) (Vastenhouw et al. 2010), reminiscent of so-called bivalent domains that have been described in embryonic stem (ES) cells. In ES cells, bivalent marks are associated with genes with later roles in differentiated lineages, but whose expression is repressed during pluripotency (Bernstein et al. 2006). Analogously, during zebrafish embryogenesis, bivalent marks are found in the promoters of lineage-specific genes that are not expressed immediately at ZGA

H3K4me3 and H3K27me3: post-transcriptional modifications of histone H3 at lysine 4 and 27, consisting of trimethylation of the epsilon amino group

Embryonic stem (ES) cells: pluripotent cells derived from the blastocyst capable of differentiating into any of the three germ layers

(Lindeman et al. 2011, Vastenhouw et al. 2010) and could allow for rapid gene activation at the appropriate time. *Xenopus* embryos also have genes that are marked by both H3K4me3 and H3K27me3, but in contrast to zebrafish, the marks do not coexist in the same cell and appear to provide temporal-spatial specificity of expression rather than bivalency (Akkers et al. 2009).

But still other genes marked by H3K4me3 are not transcribed at all (at detectable levels) and eventually lose the mark during gastrulation (Lindeman et al. 2011). Therefore, H3K4me3 may have a broader role that extends beyond the immediate transcriptional needs at ZGA and is instead involved in a larger-scale remodeling of embryonic chromatin to a nonoocyte state. In support of this model, a recent study measuring genome-wide nucleosome positioning, using micrococcal
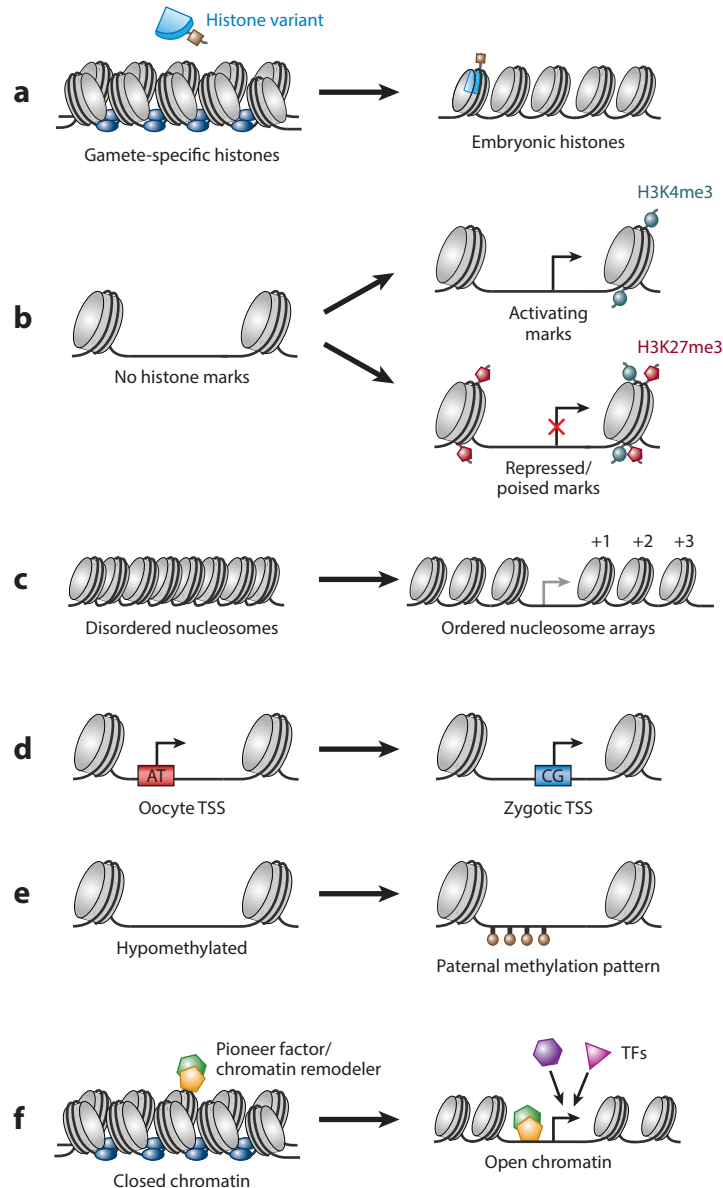
nuclease digestion (**Table 1**), found distinct patterns of occupancy on gene promoters marked by H3K4me3 (Zhang et al. 2014). At ZGA in zebrafish, well-ordered arrays of nucleosomes form, precisely positioned at the TSS of genes and independent of active transcription or Pol II binding (**Figure 5c**) (Zhang et al. 2014). These results together show that histone modifications inform the timing and spatial specificity of gene expression during the MZT, which is correlated with localized changes to chromatin conformation at zygotic genes. How this specificity is established remains largely unknown, though evidence suggests that epigenetic inheritance from the gametes plays a role.

Epigenetic Prepatterning

Epigenetic features from both the egg and the sperm chromatin appear to strongly influence the patterns of chromatin modifications acquired in the embryo. In mouse oocytes, Polycomb activity was shown to be required both for correct specification of histone marks in the embryo and for shaping the maternal contribution (Posfai et al. 2012, Puschendorf et al. 2008). Polycomb repressive complex (PRC) 1 and 2 together apply and maintain H3K27 trimethylation and repress transcription (Simon & Kingston 2009). Mouse embryos lacking the maternal contribution of Ring1 and Rnf2, two core components of PRC1, arrest at the 2-cell stage and experience aberrant gene expression at ZGA (Posfai et al. 2012). Similarly, Mll2 activity in the oocyte is required to establish H3K4me3 patterns and normal levels of zygotic gene expression, though other histone methyltransferases are likely involved (Andreu-Vieyra et al. 2010).

In both mouse and human sperm DNA, a small number of modified histones are present among the protamines, which are transmitted to the male pronucleus (Brykczynska et al. 2010, Hammoud et al. 2009). H3K4me2, H3K4me3, and H3K27me3 are found at developmental promoters in sperm, with the latter occurring preferentially at genes that are initially repressed in the embryo (Brykczynska et al. 2010, Hammoud et al. 2009). The chromatin in zebrafish sperm has also been reported to contain both H3K4me3 and H3K27me3, along with several other activating and repressing marks organized in large multivalent chromosomal regions that contain genes active late in embryogenesis (Wu et al. 2011).

However, early embryos appear to be completely devoid of distinguishing histone marks (Vastenhouw et al. 2010, Zhang et al. 2014), suggesting that these sperm modifications are initially lost but then reapplied by a mechanism that retains epigenetic memory. De novo nucleosome

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Figure 5

Modes of chromatin regulation and establishing competency for zygotic gene activation (ZGA). (*a*) Gamete-specific repressive histone variants are replaced with zygotic variants after fertilization, which often bear permissive modifications. (*b*) Selective post-transcriptional modifications of histone tails are acquired during the maternal-to-zygotic transition (MZT), including activating H3K4me3 and repressing H3K27me3 over gene promoters. Some promoters are marked bivalently (both H3K4me3 and H3K27me3), indicating a poised transcriptional state. (*c*) The organization of nucleosomes around gene promoters is associated with transcriptional competence. Ordered arrays of nucleosomes (labeled +1, +2, and +3) form during ZGA around both active promoters and promoters driving later developmental expression. (*d*) Transcription start site (TSS) use on embryonic genes shifts from oocyte-specific A/T-rich regions to G/C-rich sequences. (*e*) DNA methylation, which is associated with repression, is selectively applied during the MZT in a manner that matches the sperm methylation pattern. (*f*) Widespread compacted chromatin in the early embryo is thought to be prohibitive for transcription. Maternal pioneer transcription factors may bind closed chromatin in a sequence-specific manner and induce open chromatin through recruitment of chromatin remodelers, allowing access to gene promoters. Abbreviation: TFs, transcription factors.

repositioning and histone marks may be guided by intrinsic signals encoded by the DNA sequence. In zebrafish, a global switch in promoter usage occurs at ZGA, revealed by high-throughput cap analysis of gene expression (**Table 1**) (Haberle et al. 2014). TSS positions shift from oocyte-specific A/T-rich sequences to nearby G/C-rich regions, which are the sites of H3K4me3 marks and nucleosome repositioning prior to gene activation (**Figure 5d**) (Haberle et al. 2014). Thus, specific DNA motifs recognized by chromatin remodelers or pioneer transcription factors (see below) (**Figure 5f**) could delineate the embryonic-specific program of gene expression in a manner that aligns with sperm patterns. Recently it has been proposed that large non-coding RNAs may also play a role in epigenetic remodeling, perhaps serving as sequence-specific interfaces between chromatin modifying complexes and DNA (Guttman & Rinn 2012), though their roles during embryogenesis have only begun to be explored (Pauli et al. 2012, Ulitsky et al. 2011).

G/C-rich regions are also significant in that they are sites for 5-methylcytosine modifications, which are associated with regions of transcriptional quiescence. Cytosine methylation at CpG dinucleotides is catalyzed by DNA methyltransferases such as Dnmt1, and inhibition of methyltransferase activity causes premature expression of many zygotic genes during the MZT (**Table 2**) (Potok et al. 2013, Stancheva & Meehan 2000), though for *Xenopus* Dnmt1 only the DNA binding function independent of the actual methylation appears to be necessary to repress transcription (Dunican et al. 2008). Repressive interactions with methyl-CpG-binding domain proteins such as *Xenopus* Kaiso are necessary to regulate transcriptional timing at ZGA (Ruzov et al. 2004). Conversely, DNA hypomethylation is generally predictive of genes expressed at ZGA (Potok et al. 2013, Stancheva et al. 2002) and the deposition of H3K4me3 marks (Andersen et al. 2012), though again in *Xenopus* the relationship between methylation and gene expression may be more complex (Bogdanović et al. 2011).

As with histone marks, specific methylation patterns are present in sperm DNA (Hammoud et al. 2009, Wu et al. 2011), but these are largely lost upon fertilization, only to reappear in the blastula (Mhanni & McGowan 2004, Oswald et al. 2000, Santos et al. 2002, Smith et al. 2012, Stancheva et al. 2002). Two high-throughput assays of DNA methylation during zebrafish embryogenesis using bisulfite sequencing (**Table 1**) have revealed that nearly the exact paternal pattern of differential methylation reemerges in the blastula, despite having adopted a more oocyte-specific pattern during cleavage stages (**Figure 5e**) (Jiang et al. 2013, Potok et al. 2013). This is achieved independent of a sperm methylation template, as parthenogenic embryos produced by fertilization with UV-irradiated sperm, which lacks functional chromatin, still adopt paternal methylation patterns on chromatin derived solely from the oocyte (Potok et al. 2013), implicating intrinsic signals in the genome that guide these modifications.

In summary, transcriptional competence as well as the specificity of gene expression are achieved through regulation of chromatin accessibility in a manner informed by both parental epigenomes. The acquisition of an open nucleosome arrangement, histone modifications, and DNA methylation combine to license transcription at the MZT (**Figure 5**), though the coordination and hierarchy of these molecular events during embryogenesis remain to be elucidated. Engagement of this permissive chromatin to elicit gene expression is the role of transcriptional machinery and accessory factors, but as we describe in the final subsection, some of these factors may in fact establish accessible chromatin for themselves.

Transcriptional Repressors and Activators

During early characterizations of transcriptional competency in mouse embryos, a change in the *cis* regulatory requirements was observed from the 1- to the 2-cell stage, such that enhancer sequences became necessary for efficient transcription (Martínez-Salas et al. 1989). Sequence-specific

transcription factors that bind gene loci to recruit the transcriptional machinery may thus help navigate a repressive chromatin landscape during the major ZGA (Majumder & DePamphilis 1995).

Across all species, the availability of both general and specific transcription factors is expected to help regulate gene expression at ZGA. Components of the TFIID complex, which binds the core promoter to activate basal transcription, are one point of regulation. In *C. elegans*, TAF-4 is sequestered in the cytoplasm by phosphorylated OMA-1 and OMA-2, thus preventing TFIID from assembling onto nuclear DNA during the first cleavages (Guven-Ozkan et al. 2008). Phosphorylation is mediated by fertilization through activation of the kinase MBK-2, which interestingly is also responsible for phosphorylating other maternal proteins to mark them for degradation (Stitzel et al. 2006). Pol II inactivity is maintained until the 4-cell embryo, when OMA-1 and -2 themselves are degraded and ZGA begins (Guven-Ozkan et al. 2008).

Another TFIID subunit, TATA-binding protein (TBP), along with its paralogs TBP2 and TBP-like, was found to be essential for embryonic development in *Xenopus*, *C. elegans*, and zebrafish (Bártfai et al. 2004; Dantonel et al. 2000; Ferg et al. 2007; Jallow et al. 2004; Kaltenbach et al. 2000; Martianov et al. 2002; Müller et al. 2001; Prioleau et al. 1994; Veenstra et al. 1999, 2000), with semiredundant roles in transcription activation. In *Xenopus*, TBP is rate limiting for embryonic transcription (Prioleau et al. 1994, Veenstra et al. 1999). Although TBP mRNA is maternally provided, protein levels are undetectable until the early blastula (Veenstra et al. 1999). This is in contrast to other basal transcription factors, such as TGIIB and TFIIF RAP74 (Veenstra et al. 1999), suggesting that specific translational repression of TBP, likely mediated by delayed cytoplasmic polyadenylation, contributes to early transcriptional quiescence. In mouse, TBP loss results in failed transcriptional activation in the blastocyst, though the effect was observed only for Pol I and III transcription (Martianov et al. 2002). Additionally, in *D. melanogaster*, TBP is strongly associated with the earliest transcribed genes (Chen et al. 2013). Overall, differential TATA box usage in gene promoters coupled with post-transcriptional regulation of maternal TATA-binding factors may be a mechanism to define temporally specific patterns of activation for different sets of genes during the MZT.

Establishing critical levels of other activating and repressive transcription factors likely plays a significant role during ZGA; however, to date few such factors have been identified. In *D. melanogaster*, Tramtrack (TTK) is a maternal inhibitor of *fushi tarazu* expression (Brown et al. 1991, Pritchard & Schubiger 1996). Nuclear concentration of TTK is simultaneously reduced by an increasing N/C ratio and by regulated degradation of *ttk* mRNA by the maternal clearance factor Smaug (Benoit et al. 2009, Tadros et al. 2007). Loss of Smaug has a widespread effect on zygotic gene expression, likely beyond the effect of sustained TTK activity, suggesting that Smaug is responsible for inactivating several other unknown repressive factors that are involved in ZGA (Benoit et al. 2009, Chen et al. 2014).

Once repression is overcome, early gene activation in *D. melanogaster* is driven by Zelda (also known as Vielfältig) (Liang et al. 2008), a maternally deposited transcription factor with ubiquitous expression in the preblastoderm embryo and specific patterns of subcellular localization over the course of the cell cycle (Staudt et al. 2006). Zelda binds promoter regions of early zygotic genes via a heptamer DNA motif called the TAGteam (De Renzis et al. 2007, ten Bosch et al. 2006) that is conserved in other insects (Biedler et al. 2012). Loss of Zelda causes mitotic defects (Staudt et al. 2006) and a failure to activate 120 early zygotic genes during cycles 8–13 (Liang et al. 2008). However, Zelda has also been shown to bind hundreds of additional gene promoters and enhancers prior to their eventual activation (Harrison et al. 2011, Nien et al. 2011), suggesting a broader role in licensing zygotic transcription.

In zebrafish, early zygotic genes are enriched in binding sites for the pluripotency-inducing factors Nanog, Pou5f3 (formerly known as Pou5f1 and homologous to mammalian Oct4), and

Sox19b (a homolog of Sox2 in the SoxB1 family) (Lee et al. 2013, Leichsenring et al. 2013). These three factors are maternally provided (Burgess et al. 2002, Okuda et al. 2010, Onichtchouk et al. 2010, Schuff et al. 2012, Xu et al. 2012) and are the most highly translated sequence-specific transcription factors prior to ZGA, as determined by ribosome profiling (Lee et al. 2013). Combined loss of these factors has a profound effect on transcriptional output and development, with 82% of genes deficient in the late blastula and a corresponding failure to initiate gastrulation, similar to an α-amanitin phenotype (Lee et al. 2013). Binding of the factors appears to be coordinated and widespread (Bogdanović et al. 2012, Leichsenring et al. 2013, Xu et al. 2012), reminiscent of their homologs' behaviors in ES cells and induced pluripotent stem (iPS) cells, where they guide specific gene expression patterns to induce or maintain pluripotency (reviewed in Young 2011).

Nanog, Sox2, and Oct4 are associated with chromatin remodeling activities in ES and iPS cells (Orkin & Hochedlinger 2011) and may have pioneering activity by binding to regions of repressed chromatin to induce nucleosome repositioning and allow other factors to bind in a cooperative manner (**Figure 5f**) (Zaret & Carroll 2011). In fact, they may play similar roles during ZGA (Lee et al. 2013, Leichsenring et al. 2013) and would thus provide a mechanism by which the silent embryonic genome is initially engaged. In *Xenopus*, β-catenin has been shown to induce epigenetic modifications at genes prior to their eventual expression during early embryogenesis, implicating it as another maternal factor involved in establishing chromatin competency (Blythe et al. 2010).

The widespread binding pattern of Zelda on gene regulatory regions suggests that it too may have pioneering activity. Zelda binding is correlated with accessible chromatin, as measured by DNase I hypersensitivity (**Table 1**) (Harrison et al. 2011, Thomas et al. 2011), and TAGteam sequences are additionally bound by other factors (Satija & Bradley 2012), including Bicoid Stability Factor (De Renzis et al. 2007), STAT92E (Tsurumi et al. 2011), and Grainyhead (Harrison et al. 2010). In this way, gene activation may be the product of combinatorial binding of Zelda, which primes the chromatin, and other transcription factors, which would provide the temporal-spatial specificity and magnitude of the transcriptional output (**Figure 5f**). In sum, maternal transcription factors have widespread roles in guiding ZGA at specific gene loci. However, specific factors with pioneering activity may ultimately determine ZGA by binding repressed chromatin and inducing remodeling, thus allowing the transcriptional machinery to access gene promoters and drive zygotic gene expression.

DEVELOPMENTAL SIGNIFICANCE OF THE FIRST ZYGOTIC GENES

Functions of the First Zygotic Genes

Zygotic gene expression during the MZT provides the embryo with what the maternal contribution lacks. To some extent, these genes will encode transcription factors and signaling pathway components that prepare the embryo for gastrulation and cell specification and differentiation, but there will also be genes that carry out basic functions common to all cell types and tissues, the so-called housekeeping genes. Increased cell number, protein turnover rates, or the need to establish specific spatial expression patterns may explain why it is necessary to supplement or replenish large parts of the maternal contribution.

The relative proportion of these two classes of genes varies widely among different organisms, likely correlated with the different times at which ZGA occurs in relation to developmental milestones. In mouse, where both the minor and major waves of ZGA occur prior to two cleavages, spanning two days of development, activated genes predominantly encode basic cellular functions, including protein and RNA metabolism; transcription factors and patterning genes seem to activate slightly later (Hamatani et al. 2004, Park et al. 2013, Xue et al. 2013). In contrast, zebrafish

zygotic transcripts appear after 2–2.5 h of development supported by the maternal contribution and ~2 h prior to the onset of gastrulation; accordingly, ZGA involves roughly equal numbers of housekeeping genes and genes encoding transcription factors and chromatin modifiers (Aanes et al. 2011, Lee et al. 2013). Signaling and patterning genes appear early in *Xenopus* (Paranjpe et al. 2013, Yang et al. 2002), as well as in *D. melanogaster* (Ali-Murthy et al. 2013, Karr et al. 1985), where early genes are specifically required for proper cell-division synchrony (Karr et al. 1985), establishing the anterior-posterior axis (Blankenship & Wieschaus 2001) and cellularization (Lecuit et al. 2002).

Beyond these broad themes of gene function, there is not a strong coherence in the patterns of zygotic expression for individual genes. Among more closely related species, orthologous zygotic genes vary greatly in expression timing (Xue et al. 2013) and levels (Yanai et al. 2011), which suggests a rapidly evolving regulatory landscape (Xie et al. 2010). Across longer evolutionary distances, zygotic genes appear to be almost completely divergent (Heyn et al. 2014). As an extreme example, much of the maternal contribution in *C. elegans* is in fact transcribed de novo in fellow nematode *A. suum*, including critical regulators of the MZT, such as *oma* (Wang et al. 2014). The magnitude of gene expression divergence for such a fundamental process as ZGA seems paradoxical; however, underlying core conserved functionality likely exists and remains to be further characterized.

The Intimate Relationship between Zygotic Genome Activation and Maternal Clearance

One notable function that does seem to be more conserved, in both theme and mechanism, is maternal clearance. Two forms of maternal mRNA decay are found across animals: the maternal mode, which relies on maternally provided factors, and the zygotic mode, which depends on de novo zygotic transcription (Bashirullah et al. 1999, Walser & Lipshitz 2011). In many species, failure to properly activate zygotic gene expression leads to loss of the zygotic mode of maternal clearance and stabilization of maternal messages (**Figure 6a,b**) (Bashirullah et al. 1999, De Renzis et al. 2007, Edgar & Datar 1996, Hamatani et al. 2004, Lee et al. 2013, Surdej & Jacobs-Lorena 1998, Tadros et al. 2007).

Generally, both modes can be found in a given species. In mouse, there is a strong maternal mode that is active shortly after fertilization (Hamatani et al. 2004, Pikó & Clegg 1982), but a second round of clearance overlaps with the major ZGA and is abrogated by transcription inhibition (Hamatani et al. 2004). A prominent maternal mode is also found in *Xenopus* embryos, partially mediated by AU-rich elements (Voeltz & Steitz 1998) and embryonic deadenylation elements (EDEN) (Bouvet et al. 1994, Paillard et al. 1998) in the 3′ untranslated regions of maternal mRNAs. Fertilization-induced activation of maternal factors, including EDEN-binding protein (Paillard et al. 1998), causes deadenylation of target mRNAs (Duval et al. 1990). However, zygotic transcription is required for these deadenylated transcripts to eventually be degraded (Audic et al. 1997, Duval et al. 1990). *D. melanogaster* Smaug also induces maternal mRNA deadenylation by recruiting the CCR4/POP2/NOT-deadenylase complex (Semotok et al. 2005), but this activity likely does not depend on zygotic transcription. However, other zygotic mechanisms activate later in embryogenesis that accelerate decay for targets of the maternal mode (Bashirullah et al. 1999), in addition to destabilizing transcripts that are subject only to the zygotic mode (Surdej & Jacobs-Lorena 1998).

In the zygotic mode of clearance, microRNAs (miRNAs) play an important role. miRNAs are ~22-nt small RNAs that are incorporated into silencing complexes (miRISC) that target mRNAs to induce translation repression, deadenylation, and decay (Huntzinger & Izaurralde
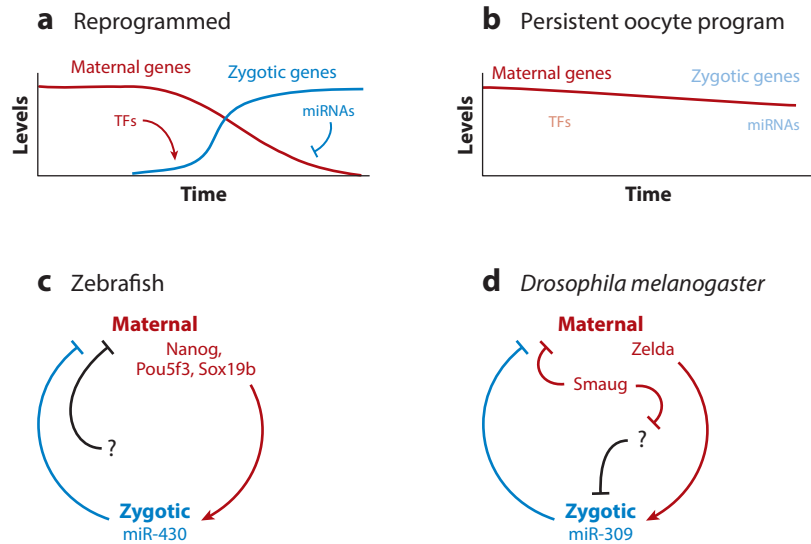
a Reprogrammed

Maternal genes   Zygotic genes

Levels

TFs   miRNAs

Time

b Persistent oocyte program

Maternal genes   Zygotic genes

Levels

TFs   miRNAs

Time

c Zebrafish

Maternal
Nanog,
Pou5f3, Sox19b

?

Zygotic
miR-430

d *Drosophila melanogaster*

Maternal
Zelda

Smaug

?

Zygotic
miR-309

Figure 6

Activation of the zygotic genome and maternal clearance together reprogram the embryo during the maternal-to-zygotic transition. (*a*) Maternally provided factors (*red*) activate transcription of zygotic genes (*blue*) through the action of transcription factors (TFs) that engage the early embryonic genome. In turn, zygotic genes, including microRNAs, direct clearance of maternal RNAs. (*b*) Failure to activate the zygotic genome by inhibiting maternal TF activity results in stabilization of the maternal program and arrested development. (*c*) In zebrafish, the TFs Nanog, Pou5f3, and Sox19b are required for widespread zygotic gene expression, which includes miR-430. miR-430, along with other unknown factors, induces clearance of a large subset of maternal RNAs. (*d*) In *Drosophila melanogaster*, Zelda plays a similar role and is responsible for activating miR-309, which in turn mediates maternal clearance. Maternal Smaug activity is also required for maternal clearance, as well as for miR-309 expression, through an unknown intermediate.

2011). The role of miRNAs in maternal clearance was first identified in zebrafish with the aid of a maternal-zygotic mutant for *dicer* (MZ*dicer*) (Giraldez et al. 2006), which encodes an RNase III enzyme required for canonical miRNA biogenesis. MZ*dicer* embryos are deficient in mature miR-430, which results in the stabilization of hundreds of maternal mRNAs that are normally translationally repressed and degraded in the wild-type embryo, comprising at least 20% of maternally contributed genes (Bazzini et al. 2012, Giraldez et al. 2006). The miR-430 gene cluster is one of the earliest and most highly transcribed genes from the zygotic genome (Chen et al. 2005, Heyn et al. 2014, Lee et al. 2013) and is directly activated by ZGA transcription factors Nanog, Pou5f3, and SoxB1 (**Figure 6c**) (Lee et al. 2013). Loss of Nanog in particular results in severely reduced miR-430 levels leading to stabilized maternal mRNAs (Lee et al. 2013), thus illustrating the tight coordination of ZGA and maternal clearance (**Figure 6**).

    miR-430 has conserved zygotic expression in another teleost fish, medaka (Tani et al. 2010), and has sequence similarity to several other embryonic miRNAs, including miR-295 in mouse; miR-302, miR-327, and miR-516-520 in human (Chen et al. 2005, Giraldez 2010, Svoboda & Flemr 2010); members of the vertebrate miR-17-92 cluster (Tang et al. 2007); and miR-427 in *Xenopus* (Lund et al. 2009, Watanabe et al. 2005). miR-427 has similar expression dynamics to miR-430 in the early *Xenopus* embryo (Watanabe et al. 2005) and was likewise shown to mediate maternal clearance activity (Lund et al. 2009), whereas miR-302 was found to be activated by Oct4 and Sox2 in ES cells (Card et al. 2008).

Beyond vertebrates, the miR-309 cluster plays an analogous role in *D. melanogaster* embryogenesis, suggesting that flies have independently evolved a similar strategy to clear maternal mRNAs (Bushati et al. 2008). miR-309 is activated by Zelda along with other early zygotic protein-coding and miRNA genes (Fu et al. 2014, Liang et al. 2008), and loss of miR-309 function leads to stabilization of ∼13% of maternal transcripts cleared in wild-type embryos (Bushati et al. 2008). Interestingly, mRNA clearance activity mediated by maternally provided Smaug is also required for normal miR-309 expression (Benoit et al. 2009) (**Figure 6d**). Thus, distinct pathways of maternal clearance may be functionally linked through the regulation of zygotic transcription (**Figure 6**).

Pioneer factor:
a transcription factor that can bind closed chromatin to induce chromatin remodeling and accessibility for other factors

CONCLUDING REMARKS

Zygotic Gene Expression at the Maternal-to-Zygotic Transition Is a Multifaceted Process

In conclusion, we have described the context, mechanisms, and function of ZGA and gene expression during the MZT, a process that is fundamentally conserved across animals and yet in many ways is surprisingly variable among different species. High-throughput transcriptome analyses show widespread, but specific, patterns of zygotic gene transcription, much of which overlaps with the maternal contribution of RNAs. Regulation of the cell cycle, chromatin, and transcriptional machinery by maternal factors together elicits transcriptional output with individual-gene specificity. Zygotic genes span the functional requirements of the developing embryo, including the clearance of maternal instructions, as the embryonic genome assumes developmental control. Despite the diversity of mechanisms found across different species to regulate gene expression, unifying themes are emerging, including the roles of key transcription factors and miRNAs in directing the MZT (**Figure 6**). We anticipate that other common regulatory paradigms will soon emerge.

Although great advances have been made in understanding the function of each of these aspects of the MZT and ZGA, we still lack a unified picture of how all of these components combine to precisely activate transcription. Does ZGA arise from the cumulative effects of several independent mechanisms, or is there a cascade of molecular events that has a single point of control? Specifically, understanding how maternal transcription factors influence the embryonic chromatin will be key to distinguishing these two scenarios, and it may be that the activity of pioneer factors is ultimately what induces genome competency and initiates the zygotic program.

We also lack a complete understanding of how zygotic gene activation and maternal clearance complement each other and feed back on themselves. Zygotic transcription is already known to be required for some maternal clearance, and vice versa, but how this is accomplished and what factors are involved are still largely unknown. It is tempting to speculate that there are additional mechanisms that directly link these two RNA metabolic activities.

Cellular Reprogramming During Embryogenesis and Beyond

We close by revisiting the idea that the MZT is a cellular reprogramming event in vivo, by deleting the old, maternal program and installing and maintaining the new zygotic program. Maternal clearance, zygotic gene expression, and the cross talk between them combine to induce a transformation in cellular identity, away from the terminally differentiated gametes and toward transient stages of totipotency, pluripotency, and eventually redifferentiation (Giraldez 2010). Aspects of this process and the mechanisms regulating it are found beyond embryogenesis, most notably in stem cells, which have simultaneous requirements to give rise to differentiated cell types and to self-renew to maintain an undifferentiated state.

iPS cells are the in vitro instantiation of this process. Reprogramming of mouse somatic cells to a pluripotent state was achieved through heterologous expression of the four so-called Yamanaka factors Oct4 (Pou5f1), Sox2, Klf4, and c-Myc (Takahashi & Yamanaka 2006). Although the mechanisms leading to induced pluripotency are poorly understood, numerous other transcription factors, chromatin modifiers, and miRNAs are known to be involved, and manipulation of many of these can enhance reprogramming or even substitute for some of the four factors (Young 2011). However, these various players all seem to lead back to a regulatory circuit consisting of Oct4, Sox2, and Nanog, which are endogenously activated during reprogramming and are at the core of the pluripotency gene network (reviewed in Oron & Ivanova 2012, Young 2011).

Core transcription factors are central to embryogenesis as well, which is already apparent in two organisms: Zelda in *D. melanogaster* and homologs of Nanog, Oct4, and Sox2 themselves in zebrafish. In this way, the postfertilization mobilization of maternal factors is analogous to the introduction of Yamanaka factors into somatic cells, with a similar end result: activation of a pluripotency network through expression of the core regulatory circuit. If iPS cells are in fact an in vitro recapitulation of the MZT, then there potentially exist many possible paths to pluripotency that will lead to the same core regulatory circuit, which may account for some of the species-specific differences that have been observed in the maternal contribution and targets of ZGA. As we continue to elucidate the mechanisms that feed into this genetic network, we will develop a greater understanding of the principles that guide cellular reprogramming both in vivo and in vitro, and more generally how gene regulation can induce large-scale changes in cellular identity.

SUMMARY POINTS

1. Zygotic gene activation and maternal clearance are conserved activities during the MZT that combine to reprogram terminally differentiated gametes to embryonic totipotency/ pluripotency.

2. Recent high-throughput interrogation of the embryonic transcriptome, coupled with dissection of the maternal and zygotic contributions, has revealed earlier and broader patterns of zygotic transcription than have been observed previously.

3. Cell cycle dynamics and chromatin structure combine to license gene expression during the MZT.

4. Maternally provided pioneer transcription factors may initiate ZGA by inducing open chromatin and recruiting other transcriptional machinery.

5. Zygotic miRNAs transcribed soon after ZGA are key players in the zygotic mode of maternal clearance across different species.

FUTURE ISSUES

1. How do broadly conserved mechanisms that regulate the MZT integrate with species-specific gene expression to direct developmental programs across different organisms?

2. What is the role of genome quiescence? Does a truly transcriptionally quiescent period exist in different species, or are we limited by current detection technologies?

3. How are maternal factors post-transcriptionally regulated during oogenesis versus during embryogenesis, and does differential regulation control the timing of ZGA?

4. What are the biochemical determinants of maternal mRNA regulation (e.g., structural elements, ribonucleotide modifications, and RNA-binding proteins)?

5. What changes to embryonic chromatin are directly required for ZGA versus instructive for gene expression beyond the MZT?

6. What is the role of pioneer transcription factors in inducing open chromatin prior to ZGA?

7. How is maternal clearance informative for ZGA, and what other mechanisms regulate maternal mRNA stability?

8. How do the mechanisms underlying the MZT induce cellular reprogramming in vitro?

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